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miniprep s ; scR-C028 in SFG.

MINIPREP FOR DNA PREPARATION

1) INOCULATE CLONES INTO 3.0 ML OF LB MEDIA, OVERNIGHT AT 37 DEG.

2] THE FOLLOWING DAY LABEL 1.5 ML STERILIZED EPPENDORF TUBES TO BE USED.

2 ml 3] FILL 3/4 OF EACH TUBE WITH THE CULTURED BACTERIA.CLOSE THE CAP

4] SPIN TUBES USING THE EPPENDORF CENTRIFUGE FOR ONE MINUTE AT 6000 RPM.
PREPARE THE POT FOR BOILING WATER

5] DECANT THE SUPERNATANT, LEAVING A LITTLE BIT IN THE TUBE.

6] RESUSPEND THE PELLET BY VORTEXING

240µl 7] ADD 120 micro ml OF STEL-T SOLUTION . MIX GINTLY BY INVERTING THE TUBES. DO NOT VORTEX.

8] KEEP THE TUBES IN BOILING WATER FOR EXACTLY 1 MINUTE AND THEN IMMEDIATELY TRANSFER THE TUBES TO A BUCKET OF ICE.
After boiling the DNA appear whitish in colour.

9] KEEP THE TUBES IN ICE FOR 5 MINUTES.

10] SPIN THE TUBES IN THE MICROFUGE FOR 5 MINUTES AT THE HIGHEST SPEED. (12 x 1000 RPM)

→ 10µl
→ 10µl
→ 20µl.

11] USING A STERILIZED TOOTHPICK PICK UP THE PRECIPITATE AND DISCARD IT, LEAVING THE SUPERNATANT BEHIND.

240µl 12] ADD 120 micro ml OF 2-PROPRANOL (same quantity as the STEL-T solution) 2-ISOPRANOL PRECIPITATES THE DNA. MIX THE TUBES BY INVERSION AND KEEP ON ICE FOR 5 MINUTES.

13] SPIN FOR 5 MINUTES IN THE MICROFUGE AT THE HIGHEST SPEED. (12 X 1000 RPM)

14] DECANT THE SUPERNATANT AND LEAVE THE TUBES TO AIR DRY IN AN INVERTED POSITION.
[To hasten this step, you may add 200 micro ml of 100% alcohol without disturbing the pellet and allow to air dry as before.)
→ discard the alcohol.

150µl 15]ONCE THE PELLET IS DRY, WHICH CAN BE MADE OUT BY THE POWDERY APPEARANCE OF THE PELLET, RESUSPEND IN 60 micro ml OF TE WITH RNAase.

WORKING SOLUTION OF TE WITH RNAase

2 micro ml of RNAase (20 mg/ml) in 1 ml of TE
16]THESE DNA CAN BE FROZEN AT -20 TEMPERATURE IF REQUIRED.

1) NotI - Digest in 30µl.

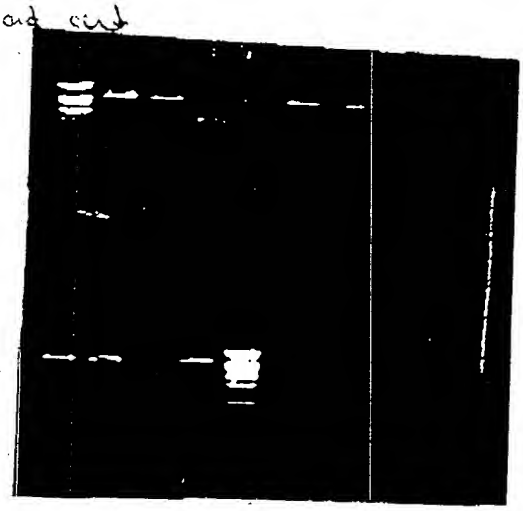
1-12 → 25µl + 3µl 10×B3 + 0.3µl 100×BSA + 1µl NotI

Mix ×14 = 42µl 10×B
3,6µl BSA 100×
14 µl NotI

TBO-HQ → 1µl + 24µl H_2O +

1 1/2h 37°C → 1% Gel, 1/10 load out

2µl ×BsteI (1µl)
3µl TBO
3µl 1-12
2µl ×BsteI (1µl)



↓
37µl
↓

2) 40µl BNI-Digest
→ 1,3µl 10×B3
1,3µl 10×BSA
1 µl BNI
9,5µl H_2O

Mix ×14 → 18.2µl 10×B3
18.2µl 10×BSA
14µl BNI
140µl H_2O
13µl/Tube
→ 2h 37°C

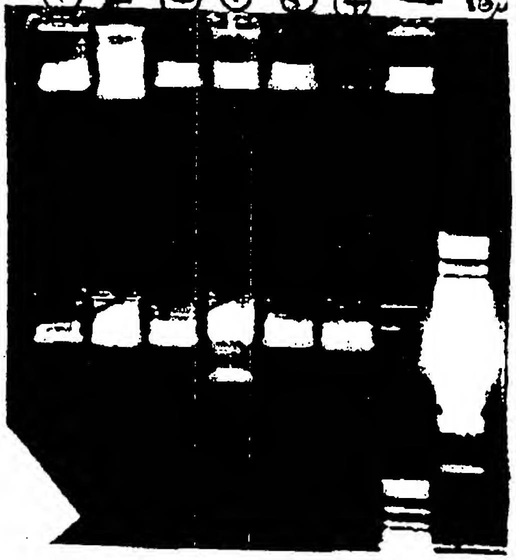
expected band ⊕ Clones 360bp (≙ PCR-fragment)
⊖ Clones 600bp → if linearized Vector was contaminating!

360bp
440

STG – Nco ScFv — NotI – CD28-TM/Cytoplasm — BHA.
STG – Nco ScFv — NotI – CD8/Zeta – BHA

~600bp

1 2 3 4 5 6 7 10µ · 1.2% Gel



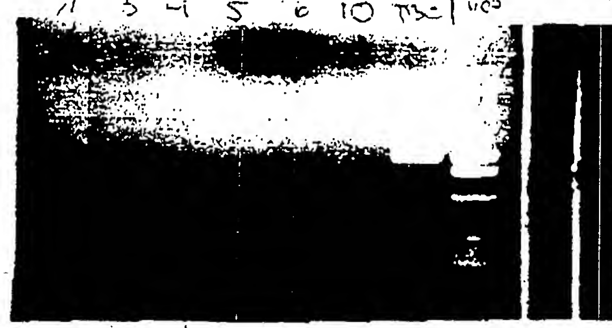
· Prep 1–7
· 10µl PBR322 (622) [8µg]
· Prep 8–12
· TBC
· 1µl PBR 322 (600) [1µg]
· 6µl λ Hind III [3µg]

1 – 7 · PBR 322

1X λ

Prep 1,3,4,5,6,10 + TBC includes digest. in 40µl / 3h
25µl DNA
4µl 10×Buffer
4µl 10×BSA ½h 37°C
4µl H2O
1µl Not
1µl BsiW

1 3 4 5 6 10 TBC | PBR322 1.5% Gel



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